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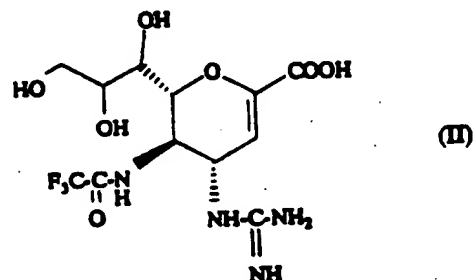
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(54) Title: DERIVATIVES OF 2-DEOXY-2,3-DEHYDRO-N-ACETYLNEURAMINIC ACID (DANA)

(57) Abstract

The compound of formula (II) and its physiologically acceptable derivatives and solvates are inhibitors of viral neuraminidase and of influenza virus. Pharmaceutical compositions and methods of treatment are also claimed.



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DERIVATIVES OF 2-DEOXY-2,3-DEHYDRO-N-ACETYLNEURAMINIC  
ACID (DANA)

This invention relates to a chemical compound and to its use in medicine. In particular the invention concerns a novel  $\alpha$ -D-neuraminic acid derivative, methods for its preparation, pharmaceutical formulations containing it and its use as an antiviral agent.

Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many micro-organisms. These include bacteria such as *Vibrio cholerae*, *Clostridium perfringens*, *Streptococcus pneumoniae*, and *Arthrobacter sialophilus*, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

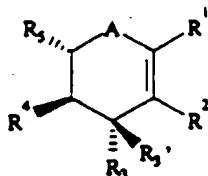
Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and its derivatives. See, e.g. Meindl et al, Virology, 1974 58 457-63. The most active of these is 2-deoxy-2,3-dehydro-N-trifluoroacetyl-neuraminic acid (FANA), which inhibits multi-cycle replication of influenza and parainfluenza viruses in vitro. See Palese et al, Virology, 1974 59 490-498.

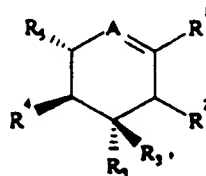
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## International Application Publication

No. WO 91/16320 describes a number of analogues of DANA of the general formulae (I) and (Ia):



(I)



(Ia)

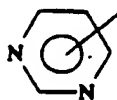
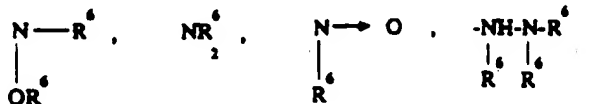
where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

R<sup>1</sup> denotes COOH, P(O)(OH)<sub>2</sub>, NO<sub>2</sub>, SOOH, SO<sub>3</sub>H, tetrazol, CH<sub>2</sub>CHO, CHO or CH(CHO)<sub>2</sub>,

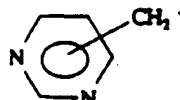
R<sup>2</sup> denotes H, OR<sup>6</sup>, F, Cl, Br, CN, NHR<sup>6</sup>, SR<sup>6</sup> or CH<sub>2</sub>X, wherein X is NHR<sup>6</sup>, halogen or OR<sup>6</sup> and

R<sup>6</sup> is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an NO<sub>2</sub> group, an NH<sub>2</sub> group or a COOH group,

R<sup>3</sup> and R<sup>3'</sup> are the same or different, and each denotes hydrogen, CN, NHR<sup>6</sup>, N<sub>3</sub>, SR<sup>6</sup>, =N-OR<sup>6</sup>, OR<sup>6</sup>, guanidino,



or



R<sup>4</sup> denotes NHR<sup>6</sup>, SR<sup>6</sup>, OR<sup>6</sup>, COOR<sup>6</sup>, NO<sub>2</sub>, C(R<sup>6</sup>)<sub>3</sub>, CH<sub>2</sub>COOR<sup>6</sup>, CH<sub>2</sub>NO<sub>2</sub> or CH<sub>2</sub>NHR<sup>6</sup>, and

R<sup>5</sup> denotes CH<sub>2</sub>YR<sup>6</sup>, CHYR<sup>6</sup>CH<sub>2</sub>YR<sup>6</sup> or CHYR<sup>6</sup>CHYR<sup>6</sup>CH<sub>2</sub>YR<sup>6</sup> where Y is O, S, NH or H, and successive Y moieties in an

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$R^5$  group are the same or different, and pharmaceutically acceptable salts or derivatives thereof, provided that in general formula (I)

(i) when  $R^3$  or  $R^3'$  is  $OR^6$  or hydrogen, and  $\lambda$  is oxygen or sulphur, then said compound cannot have both

(a) an  $R^2$  that is hydrogen and

(b) an  $R^4$  that is NH-acyl, and

(ii)  $R^6$  represents a covalent bond when  $Y$  is hydrogen, and that in general formula (Ia),

(i) when  $R^3$  or  $R^3'$  is  $OR^6$  or hydrogen, and  $\lambda$  is nitrogen, then said compound cannot have both

(a) an  $R^2$  that is hydrogen, and

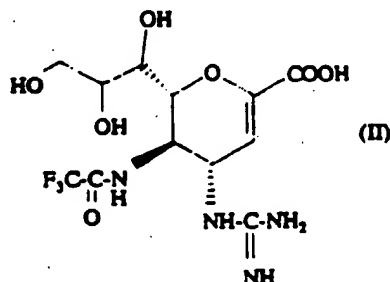
(b) an  $R^4$  that is an NH-acyl, and

(ii)  $R^6$  represents a covalent bond when  $Y$  is hydrogen

As indicated in WO 91/1630, compounds of the above formulae are active both *in vitro* and *in vivo* against viral neuraminidase and are useful in the treatment of influenza.

We have now found a particular compound which falls within the scope of the group of compounds described and claimed in WO 91/16320, but which is not specifically disclosed therein, which compound has particularly advantageous properties.

The present invention accordingly provides 2,6-Anhydro-4-guanidino-3,4,5-trideoxy-5-trifluoroacetamido-D-glycero-D-galacto-non-2-enonic acid (4-G-Neu5 F<sub>3</sub>Ac2en) of formula (II)



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and its physiologically acceptable derivatives and solvates (e.g. hydrates)

By "a pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, ester, or salt of such ester, of the compound of formula (II) or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) the compound of formula (II) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compound of formula (II) may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compound of formula (II). Of particular interest as such derivatives are compounds modified at the carboxyl function, hydroxyl functions or at amino groups. Thus compound of interest include alkyl (such as methyl, ethyl or propyl e.g. isopropyl) or aryl (e.g. phenyl, benzoyl) esters and acetyl esters of the compound of formula (II).

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compound of formula (II) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compound of formula (II) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable may be useful in the preparation of salts useful as intermediates in obtaining the compound of the invention and its pharmaceutically acceptable acid addition salts.

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Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and  $NR_4^+$  (where R is  $C_{1-4}$ alkyl) salts.

References hereinafter to the compound of the invention include the compound of formula (II) and pharmaceutically acceptable derivatives thereof.

The compound of formula (II) possesses antiviral activity. In particular this compound is an inhibitor of viral neuraminidase of orthomyxoviruses and paramyxoviruses in particular neuraminidase, for example the viral neuraminidase of influenza A and B, parainfluenza, mumps and Newcastle disease.

There is thus provided in a further aspect of the invention the compound of formula (II) or a pharmaceutically acceptable derivative thereof for use as an active therapeutic agent in particular as an antiviral agent for example in the treatment of orthomyxovirus and paramyxovirus infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, for example orthomyxovirus and paramyxovirus infections in a mammal including man comprising administration of an effective amount of the compound of formula (II) or a pharmaceutically acceptable derivative thereof.

There is also provided in a further or alternative aspect use of the compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of the compound of the invention required for use in treatment will vary with the route of administration, the nature of the condition being treated and the age and condition of the patient and will ultimately be at the

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discretion of the attendant physician or veterinarian. In general however a suitable dose will be in the range of from about 0.1 to 750mg/kg of bodyweight per day, preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compound is also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given 1-4 times daily and continued for 3-7, e.g. 5 days post infection.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage form for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, the compound of the invention may be administered as the raw chemical it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising the compound of formula (II) or a pharmaceutically acceptable derivative thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous)



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administration r in a form suitable for administration to the respiratory tract (including the nasal passages) for example by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compound according to the invention may also be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or

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dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile,  
5 pyrogen-free water, before use.

For topical administration to the epidermis the compound according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with  
10 an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening  
15 agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in  
20 an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most  
25 preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and  
30 shaping in moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to  
35 be appropriate.

For administration to the respiratory tract (including intranasal administration) according to the

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method of the invention the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for administration to the respiratory tract.

5           Thus in general the compounds may be administered in the form of a solution or a suspension or as a dry powder.

          Solutions and suspensions will generally be aqueous for example prepared from water alone (for example sterile or pyrogen-free water) or water and a  
10           physiologically acceptable co-solvent (for example ethanol, propylene glycol, polyethylene glycols such as PEG 400).

          Such solutions or suspensions may additionally contain other excipients for example preservatives (such as  
15           benzalkonium chloride), solubilising agents/surfactants such as polysorbates (e.g. Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain  
20           suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

          Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be  
25           provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be  
30           achieved for example by means of a metering atomising spray pump.

          Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable  
35           propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluoroethane, carbon dioxide or other

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suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g. gelatin or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the compound will generally have a small particle size for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired, formulations adapted to give sustained release of the active ingredient may be employed.

The compound of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compound of the invention may be employed with other antiviral agents. The invention thus provides in a further aspect a combination comprising the compound of formula (II) or a pharmaceutically acceptable derivative thereof together with another therapeutically active agent, in particular an antiviral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

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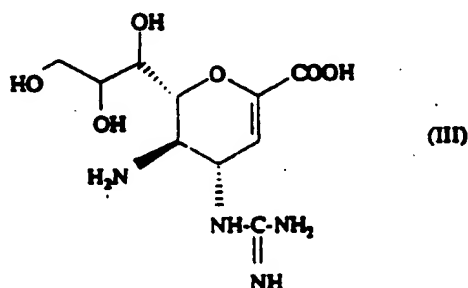
Suitable therapeutic agents for use in such combinations include other anti-infective agents, in particular anti-bacterial and anti-viral agents such as those used to treat respiratory infections. For example, other compounds effective against influenza viruses, such as amantadine, rimantadine and ribavirin, may be included in such combinations.

The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compound of the invention is used with a second therapeutic agent active against the same virus the dose of each compound may either be the same as or differ from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compound of formula (II) and its pharmaceutically acceptable derivatives may be prepared by any method known in the art for the preparation of compounds of analogous structure. In particular the compounds of formula (II) may be prepared by the methods described below.

The compound of formula (II) may be prepared from the compound of formula (III) (4-guanidinoNeu2en)



by treatment with a trifluoroacylating agent in the presence of a base.

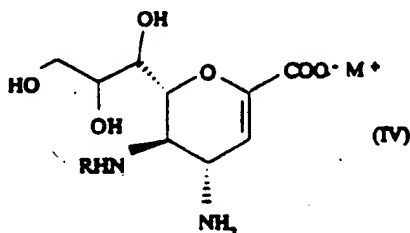
Suitable trifluoroacylating agents of use on the reaction include methyl trifluoroacetate. Suitable bases

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of use in the reaction include tertiary amines such as, for example, triethylamine.

The reaction is conveniently effected in a suitable organic solvent, such as an alcohol, for example, methanol.

The compound of formula (III) may be prepared from the compound of formula (IV)

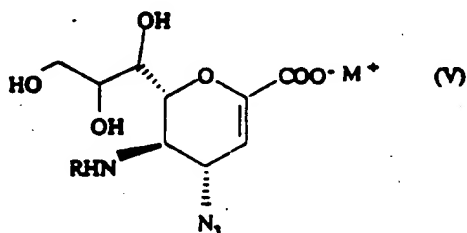


wherein  $M^+$  represents an alkali metal cation, such as  $Na^+$ , and R represents a protecting group, such as a t-butoxycarbonyl (Boc) group, by treatment with a reagent suitable to introduce the guanidino function, followed by treatment with an acid, and subsequent deprotection if necessary.

Reagents suitable to introduce the guanidino function include S-methylisourea and aminoguanidinesulphonic acid in the presence of a base such as an alkali metal carbonate, for example potassium carbonate.

Suitable acids include organic acids such as, for example, trifluoroacetic acid. Conveniently the protecting group R is so chosen that it is removed in the acidification step.

The compound of formula (IV) may be prepared from the aside of formula (V)

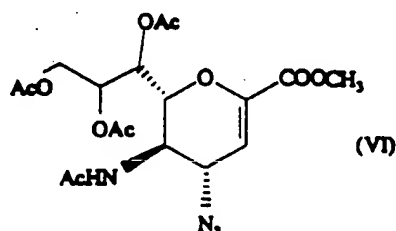


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wherein R and M' are as previously defined, by reduction.

The reduction is conveniently effected using triphenylphosphine in a suitable solvent such as pyridine, dimethylformamide, or a mixture thereof.

- 5           The compound of formula (V) may be prepared by conventional methods from a suitably protected analogue, such as the compound of formula (VI) (4-azido-Neu5,7,8,9-Ac<sub>2</sub>enlMe)



- 10           The preparation of the compound of formula (VI) is described in WO 91/16320.

- The protecting groups used in the preparation of compounds of formula (I) may be used in conventional manner. See for example "Protective Groups in Organic Chemistry" Ed. J.F.W. McOmie (Plenum Press 1973) or  
 15           "Protective Groups in Organic Synthesis" by T.W. Greene and P.G.M. Wuts (John Wiley and Sons 1991).

- Where it is desired to isolate the compound of the invention as a salt, for example as an acid addition salt, this may be achieved by treating the free base of  
 20           general formula (I) with an appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

- The present invention is further described by the following examples which are for illustrative purposes only  
 25           and should not be construed as a limitation of the invention.

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Example 1      2,6-Anhydro-4-guanidino-3,4,5-trideoxy-5-trifluoroacetamido-D-glycero-D-galacto-non-2-enonic acid

To methyl 5-acetamido-tri-O-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonate (4-azido-Neu5,7,8,9Ac,2en1Me) (10g) in 1,4-dioxan (100ml) was added di-tert-butyl dicarbonate (9.55g) and 4-dimethylamino pyridine (500mg). After 72 hours the solvent volume was reduced by approximately two thirds and a second aliquot of di-tert-butyl dicarbonate (3g) added. Seventeen hours later, a third batch of di-tert-butyl dicarbonate (3g) was added and the reaction heated at 80°C for 2 hours. The product was isolated by removing the dioxan in vacuo and chromatographing the resulting oil on flash silica gel (Merck 9385) with ethyl acetate/cyclohexane (2:3) to yield (4S,5R,6R)-5-[acetoxy-(t-butoxycarbonyl)-amino]-4-azido-6-[(1S,2R)-1,2,3-triacetoxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester as an orange oil (10.46g).  
 $^1\text{H}$  nmr (DMSO-d<sub>6</sub>) 5.99 (br.s, 1H), 5.27-5.15 (m, 2H), 4.95-4.65 (m, 3H), 4.5 (m, 1H), 4.15-4.02 (m, 1H), 3.75 (s, 3H), 2.35 (s, 3H), 1.99 (s, 9H), 1.55 (s, 9H); MS M/Z 574 MNH<sup>+</sup>, 474 MNH<sup>+</sup>, - BOC

4-Azido-Neu5,7,8,9Ac,5Boc2en1Me (180 mg) was dissolved in anhydrous methanol (25 mL) containing sodium methoxide (26mg). The mixture was stirred at room temperature for 3 h before it was evaporated to dryness. The resulting residue was stirred in 0.1M sodium hydroxide solution (10mL) at room temperature for 4h. the solution was then adjusted to pH7 with Dowex-50W X 8 (H<sup>+</sup>) resin and filtered. The filtrate was evaporated to dryness to afford 4-azido-Neu5Boc2en (120mg) as a light brownish solid. i.r. (KBr) cm<sup>-1</sup>, 3400 (br), 2980, 2930, 2100 (M<sub>2</sub>), 1690, 1600 (br);  $^1\text{H}$ -NMR (D<sub>2</sub>O): (ppm) 1.36 (s, 9H), 3.56 (dd, 1H, J 6.2 Hz, J' 12.1 Hz), 3.65 (d, 1H, J 9.3 Hz), 3.70 - 3.92 (m, 3H), 4.17 (d, 1H, J 11.1 Hz) 4.22 (d, 1H, J 8.2 Hz), 5.60 (br, s, 1H); FABMS: 397 (M+1)<sup>+</sup>.



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T a solution of 4-azido-Neu5Boc2en (120mg) in a mixture of dimethylformamide (6mL) and pyridine (12mL), was added triphenylphosphine (160 mg) at room temperature. The mixture was stirred under argon at room temperature for 2h, and then evaporated under high vacuum to dryness. The residue was stirred in methanol (10mL) at room temperature for 1h, then vacuum evaporated to dryness. The resulting solid residue was chromatographed (silica gel, ethyl acetate/2-propanol/water = 5/2/1) to give a crude product which was partitioned between water (20mL) and ethyl acetate (20mL). The aqueous phase was vacuum evaporated to dryness. The residue was chromatographed (silica gel, ethyl acetate/2-propanol/H<sub>2</sub>O = 10/2/1, then ethyl acetate/2-propanol/H<sub>2</sub>O = 4/2/1) to afford an off-white solid 4-amino-Neu5Boc2en (54mg). i.r. (KBr) cm<sup>-1</sup>, 3400 (br), 2900 - 3000, 1710, 1610 (br); <sup>1</sup>H-NMR data (D<sub>2</sub>O): (ppm), 1.31 (s, 9H), 3.51 (br.d, 1H, J 9.2 Hz), 3.56 (br.dd, 1H, J 5.6 Hz, J' 12.4Hz), 3.70 - 3.82 (m, 3H), 3.86 (br.d, 1H, J 9.2Hz), 3.98 (br.d, J 9.4 Hz), 5.60 (br.s, 1H); FABMS: 371 (M+1)<sup>+</sup>.

To a well-stirred solution of 4-amino-Neu5Boc2en (50mg) in water (5mL), were added aminoiminomethanesulfonic acid (170mg) and potassium carbonate (194mg) over a period of 8h at a reaction temperature of 35°C to 40°C. The mixture was allowed to stand at room temperature overnight, then diluted with water (10mL), and subsequently filtered. The filtrate was neutralized with 1M HCl to pH 6, then vacuum evaporated to dryness. The residue was stirred in trifluoroacetic acid (2mL) at room temperature for 2h before it was concentrated to remove trifluoroacetic acid. The resulting residue was partitioned between water (10mL) and ethyl acetate (10mL). The aqueous layer was washed with fresh ethyl acetate (5mL), then evaporated to dryness. The residue was dissolved in water (10mL) and then passed through a column of Amberlite IR-120 (H<sup>+</sup>) resin (10mL). The column was washed with water (30 mL), and then the resin was eluted with a 0.2 M-1.0 M gradient of ammonium

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hydroxide solution. The eluate which was positive towards both ninhydrin and Sakaguchi reagents was collected, evaporated to dryness, and then freeze-dried to afford 4-guanidino-Neu2en as a white solid (5mg). i.r. (KBr)  $\text{cm}^{-1}$  3370 (br), 1680, 1600 (br), 1410, 1090;  $^1\text{H-NMR}$  data ( $\text{D}_2\text{O}$ ) (ppm), 2.96 (dd, 1H, J 9.6 Hz, J' 10.6 Hz), 3.61 (dd, 1H, J 5.8 Hz, J' 11.7 Hz), 3.80 (br.d, 1H, J 9.5Hz), 3.82 (dd, 1H, J 2.6Hz, J' 11.7Hz), 3.87 (m, 1H), 3.94 (br d, 1H, J 10.6 Hz), 4.10 (dd, 1H, J 1.9 Hz, J' 9.6 Hz), 5.46 (d, 1H, J 1.9 Hz);  $^{13}\text{C NMR}$  data ( $\text{D}_2\text{O}$ ) (ppm). 52.7, (C-5), 56.9 (C-4), 67.4 (C-9), 70.4 (C-8), 82.3 (C-6), 108.7 (C-3), 153.9 (C-2), 162.3 (C-10) 173.7 (C-1); FABMS: 291 ( $\text{M}^+ + 1$ ).

To a stirred solution of 4-guanidinoNeu2en (50mg) in methanol (50 mL) were added methyl trifluoroacetate (0.5 mL) and triethylamine (0.28 mL) at room temperature over a period of 8h. The resulting solution was allowed to stir at room temperature for 3 days, then vacuum evaporated to dryness. The residue was subjected to flash chromatography (silica gel, 2-propanol/ $\text{H}_2\text{O}$  = 3/1). The fractions with  $R_f$  of about 0.7 were collected and vacuum concentrated to dryness. The residue was treated with 95% 2-propanol/ $\text{H}_2\text{O}$  solution (10mL) to give a white crystal of 4-guanidino-Neu5F<sub>3</sub>Ac2en (20 mg) CZE >96.5% purity. i.r. (KBr)  $\text{cm}^{-1}$  3470 (br), 1710, 1650 (br), 1430, 1220;  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ ) (ppm), 3.52 (br.d, 1H, J 9.2 Hz), 3.54 (dd, 1H, J 6.3 Hz J' 11.9 Hz), 3.79 (dd, 1H, J 2.3Hz, J' 11.9 Hz), 3.85 (ddd, 1H, J 2.3 Hz, J' 6.3 Hz, J'' 9.2 Hz), 4.25 (dd, 1H, J 9.4 Hz, J' 10.6 Hz), 4.39 (br.d, 1H, J 10.6 Hz), 4.49 (dd, 1H, J 1.6 Hz, J' 9.4 Hz), 5.53 (d, 1H, J 1.6 Hz);  $^{13}\text{C NMR}$  ( $\text{D}_2\text{O}$ ) (ppm), 48.7 (C-5), 50.8 (C-4), 63.0 (C-9), 68.1 (C-7), 69.8 (C-8), 74.9 (C-6), 103.6 (C-3), 149.5, 151.2, (C-2 & C-11), 157.1 (C-10), 168.8 (C-1); FABMS: 387 ( $\text{M}^+ + 1$ ). Anal Calcd for  $\text{C}_{15}\text{H}_{17}\text{F}_3\text{O}_4\text{N}_4 \cdot 3\text{H}_2\text{O}$ : C, 32.7; H, 5.2; N, 12.7. Found C, 32.5; H, 4.6; N, 12.3.

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**Example 2**      **Inhibition on Neuraminidase Activity**

5      The ability of the compound of the invention to inhibit the activity of neuraminidase *in vitro* was determined using the method described in our earlier International Application Publication No. WO 91/16320. Using this method, the compound was found to be a slow binding inhibitor of neuraminidase from both influenza A and influenza B, having  $K_i$  of approximately  $10^{-9}$  M.

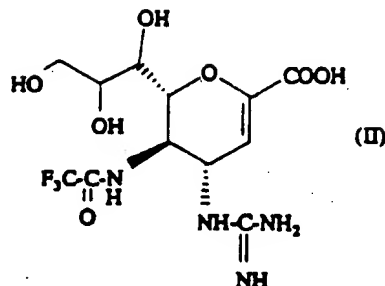
**Example 3**      **Inhibition of Influenza Virus**

10      The ability of the compound of the invention to inhibit to multiplication of influenza virus was determined using the method described in WO 91/16320. In this plaque reduction assay, the Plaque  $ISD$  for influenza A was 0.03, and for influenza B was 0.01.

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CLAIMS:

1. The compound of formula (II)



and physiologically acceptable derivatives and solvates thereof.

2. A compound as claimed in Claim 1 for use in therapy.
3. The use of a compound as claimed in Claim 1 for the manufacture of a medicament for the treatment of a viral infection.
4. A method of treatment of a viral infection in a mammal which method comprises administration to said mammal of an effective amount of a compound as claimed in Claim 1.
5. A method according to Claim 4, wherein the mammal is a human.
6. A method according to Claim 4, wherein the compound is administered to the respiratory tract.
7. A method according to any one of Claims 4 to 6, wherein the virus is influenza virus.
8. A pharmaceutical composition comprising a compound as claimed in Claim 1 and a pharmaceutically acceptable carrier therefor.
9. A pharmaceutical composition as claimed in Claim 8 adapted for administration to the respiratory tract.
10. A process for the preparation of a compound as claimed in Claim 1, which process comprises reaction of 5-amino-2,6-anhydro-4-guanidino-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid (4-guanidino-Neu2en) or a protected derivative thereof, with a trifluoroacetylating agent in the presence of a base.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/AU 95/00033

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl. <sup>6</sup> C07D 309/28, A61K 31/35.  According to International Patent Classification (IPC) or to both national classification and IPC												
<b>B. FIELDS SEARCHED</b>  Minimum documentation searched (classification system followed by classification symbols) IPC C07D 309/28  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU: IPC as above  Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) Derwent: C07D 309/28, JAPIO: C07D 309/28, CAS-ONLINE.												
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.										
X,Y	AU, 27242/92, A1 (BIOTA SCIENTIFIC MANAGEMENT PTY LTD) 29 April 1993. Pages 4 to 7, claims particularly page 4 line 26 and claim 3.	1-10										
X,Y	AU, 77590/91, A1 (BIOTA SCIENTIFIC MANAGEMENT PTY LTD) 11 November In particular page 4 lines 5-7, page 6 lines 1-27, claim 17 (& AU 75338/91, A1, page 6 claim 17).	1-10										
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.												
* Special categories of cited documents : <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of theory underlying the invention</td> </tr> <tr> <td>"E" earlier document but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"A" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of theory underlying the invention	"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"A" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of theory underlying the invention											
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"A" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 3 May 1995		Date of mailing of the international search report 10 MAY 1995 ( 1 0 . 0 5 . 95 )										
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929		Authorized officer G. HEARDER Telephone No. (06) 2832553										

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 95/00033

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
X,Y	Journal of Medical Chemistry, 1994, Vol. 37, pages 616-624, 1994 (American Chemical Society), M. Von Itzstein et al.; "Molecular Modeling Studies on Ligand Binding to Sialidase from Influenza virus and the mechanism of catalysis". (& Abstract published 15 January 1994 in Advance ACS Abstracts) whole document	1-10
X,Y	Glycoconjugate Journal, (1993) Vol. 10: pages 40-44, 1993 (Chapman and Hall), C.T. Holzer et al.; "Inhibition of sialidases from viral, bacterial and mammalian sources by analogues of 2-deoxy-2, 3-didehydro-N-acetyl neuraminic acid modified at the C-4 position". whole document	1-10
X,Y	Trends in Glycoscience and Glycotechnology, Vol. 5 No. 24. (July 1993) pages 309-310, 1993 (Forum: Carbohydrates Coming of Age), M. Von Itzstein et al.; "From X-ray Crystallography to Cure of Flu?". whole document	1-10
X,Y	Antimicrobial Agents and Chemotherapy, July 1993, Vol. 37, No.7, pages 1473-1479, 1993 (American Society for Microbiology), J.M. Woods et al.; "4-Guanidino-2, 4-dideoxy-2, 3-dehydro-N-acetylneuraminic acid is a highly effective inhibitor both of the Sialidase (Neuraminidase) and of growth of a wide range of Influenza A and B viruses in vitro". whole document	1-10
X,Y	Nature, Vol. 363, No. 6428, page 418-423, 3 June 1993, 1993 (Macmillan Magazines), M. Von Itzstein et al.; "Rational design of potent sialidase-based inhibitors of influenza virus replication". whole document	1-10
P,X,Y	Antiviral Research, Vol. 25 (1994) pages 123-131, May 1994 (Elsevier Science B.V.), F.G Hayden et al.; "Anti-influenza virus activity of the neuraminidase inhibitor 4-guanidino-Neu5Ac2en in cell culture and in human respiratory epithelium". whole document	1-10
P,X,Y	Antimicrobial Agents and Chemotherapy, October 1994, pages 2270-2275, (American Society for Microbiology), D.M.Ryan et al.; "Inhibition of Influenza Virus replication in mice by GG167 (4-guanidino-2, 4-dideoxy-2, 3-dehydro-N-acetylneuraminic acid) is consistent with extracellular activity of viral neuraminidase (sialidase)". whole document	1-10
P,X,Y	Antiviral Research Vol. 24 (1994) pages 351-356, 1994 (Elsevier Science B.V.), G.P. Thomas et al.; "Inhibition of the growth of influenza viruses in vitro by 4-guanidino-2, 4-dideoxy-N-acetylneuraminic acid". whole document	1-10
P,X,Y	Biochemistry and Molecular biology International, Vol. 32, No. 5, April 1995, pages 851-858, (Academic Press Australia), M.S. Pegg et al.; "slow-binding inhibition of sialidase from influenza virus". whole document	1-10
P,X,Y	Carbohydrate Research, Vol. 259 (1994) pages 301-305, 1994 (Elsevier Science B.V.), M. Von Itzstein et al.; "The synthesis of 2, 3-didehydro-2, 4-dideoxy-4-guanidinyl-N-acetylneuraminic acid: a potent influenza virus sialidase inhibitor". whole document	1-10

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/ AU 95/00033

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member	
AU, A, 27242/92	CA, A, 2081068 JP, A, 6-025209.	EP, A, 539204
AU, A, 77590/91	AP, A, 249 AU, B, 654815 CS, A, 9101145 FI, A, 924790 HU, A, 61989 JP, T, 5-507068 PT, A, 97460 WO, A, 9116320 AP, A, 9100253.	AU, A, 75338/91 CN, A, 1057260 EP, A, 526543 HU, A, 9203180 IL, A, 97936 NO, A, 923944 US, A, 5360817 ZA, A, 9103086
END OF ANNEX		